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In-Vitro Sub-Acute Oral Acetamiprid Toxicity on Hematological Indices in *Rattus norvegicus*

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Abstract

The present investigation on hematological profile was conducted on acetamiprid toxicity in rats randomly divided into four groups each having 18 healthy rats of six weeks old. Acetamiprid, a neonicotinoid insecticide was administered orally to female wistar rats at 25, 100 and 200 mg/kg body weight in group II, group III, and group IV respectively for consecutive 28 days. The rats of group I served as control. The hematological parameters were affected in the rats of all treated groups at 25, 100 and 200 mg/kg body weight as compared to control group of animals. The study suggested that this compound of the neonicotinoid group of insecticide have adverse effect on hemopoietic organs in animal through sub-acute exposure.
Key words : Acetamiprid, Wistar rats, Sub-acute toxicity, Hematology.

Pesticides are the substance that are used to prevent, repel, or destroy pests-organisms that compete for food supply, adversely affect comfort, or endanger human health (1). Pesticides are ubiquitous in the environment. They are found in food, water, homes, schools, workplaces, lawns, and gardens. Pesticides have significant economic, environmental, and public health impacts. Pesticide usages help improve human and animal nutrition through greater availability, longer storage life, and lower cost of food (2). Pesticides also assist in control of food-borne and vector-borne diseases which affect millions of children and adults and thousands annually (3). Pesticides also pose human health concerns because they are toxic substances and widely released into environment. Although the toxic actions of pesticides are targeted at specific pest species, the potential for adverse health effects in human and other nontargeted species is incompletely characterized (2). The increased use of pesticide resulted in toxicity in different species and could affect various functions like neurological, hematological, biochemical and reproductive function etc. in the body. Studies on toxicological aspect of insecticides are always useful for the rational treatment and prediction of risk of toxicity. Acetamiprid, a member of the neonicotinoid insecticide family, is a fairly new insecticide that has recently entered the market place. It offers control against many important pests that had previously evolved resistant strains to most insecticides. Acetamiprid is selective and provides outstanding control of sucking pests such as aphids and white flies. Also, laboratory and greenhouse testing revealed acetamiprid's ovicidal activity against many pest species, including the bollworm, *Helicoverpa zea* (Boddie). Dissemination of pesticides into environment has necessitated accurate identification of their potential hazards to animal and human health. Therefore, the present study was undertaken to assess the sub-acute toxicity of acetamiprid on animal health hazards and to investigate the impact of acetamiprid 20% suspension on the hematological indices in female wistar rats through sub-acute oral exposure.

Methods

Female wistar rats obtained from animal house, College of Veterinary Science and Animal Husbandry, Durg, were used in this experiment. Experimental protocol was approved by institutional ethics committee before starting of experiment. Animals were allowed to acclimatize for a period of 7 days prior to experiment and provide standard feed (Nutri lab, rodent feed, Vetcare Pvt. Ltd, Bangalore) and allowed to ad

Table 1. Effect of sub-acute acetamiprid toxicity on hematological parameters in fermale wistar rats (n=6). Values indicate Mean ± SE; superscripts may read row wise for comparison of means. Similar superscripts showing means do not differ significantly ($P \leq 0.05$), (**$P \leq$ 0.01).

Parameters	Experimental groups			
	Group I	Group II	Group III	Group IV
TEC ($\times 10^6$/cu.mm)	8.78 ± 0.195^{a}	8.45 ± 0.361^{ab}	6.87 ± 0.261^{c}**	7.65 ± 0.409^{bc}
PCV (%)	42.50 ± 1.727^{a}	44.16 ± 0.945^{a}	44.00 ± 0.577^{a}	45.83 ± 2.150^{a}
Hb (g/dl)	13.68 ± 0.248^{ab}	12.23 ± 0.221^{c}**	13.23 ± 0.320^{b}	14.13 ± 0.168^{a}
MCHC (g/dl)	32.35 ± 0.808^{a}	27.76 ± 0.759^{b}**	30.10 ± 0.808^{ab}	31.10 ± 1.167^{a}
MCV (fl)	48.37 ± 1.363^{b}	52.60 ± 1.803^{b}	64.63 ± 3.250^{a}**	60.64 ± 3.740^{a}**
MCH (pg)	15.61 ± 0.374^{b}	14.57 ± 0.520^{b}	19.44 ± 1.063^{a}**	18.81 ± 1.311^{a}
TLC ($\times 10^3$/cu.mm)	6.22 ± 0.153^{a}	6.48 ± 0.176^{a}	6.42 ± 0.539^{a}	5.23 ± 0.132^{b}**
Neutrophil (%)	20.95 ± 0.579^{c}	23.17 ± 0.703^{b}	27.21 ± 0.476^{a}**	26.17 ± 0.679^{a}**
Lymphocyte (%)	71.57 ± 0.518^{a}	67.17 ± 0.477^{b}**	63.75 ± 0.655^{c}**	64.00 ± 1.183^{c}**
Eosinophil (%)	2.45 ± 0.358^{a}	3.33 ± 0.557^{a}	3.07 ± 0.266^{a}	3.33 ± 0.333^{a}
Monocyte (%)	4.53 ± 0.435^{a}	5.83 ± 0.477^{a}	5.65 ± 0.655^{a}	5.67 ± 0.421^{a}
Basophil (%)	0.50 ± 0.341^{a}	0.33 ± 0.210^{a}	0.50 ± 0.341^{a}	0.33 ± 0.210^{a}

libitum water. Animals were randomly divided in four groups each having 18 healthy rats. Group I (control) received vehicle (normal saline) in which the acetamiprid was suspended, group II received acetamiprid at 25 mg/kg, group III and group IV received acetamiprid at 100 and 200 mg/kg body weight respectively. The female rats were administered at the above respective dose level of insecticide daily for 28 days. Hematological indices estimated using whole blood of six animal from each group on day 28 (blood was collected from retro-orbital plexus puncture method using ether anaesthetic for mild anaesthetia) (4) were as follows : Hemoglobin (Hb), Total erythrocyte count (TEC), Total leucocyte count (TLC), Packed cell volume (PCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Mean corpuscular volume (MCV), and Differential leukocyte count (DLC) or relative count.

Hematological studies were carried out on the day of collection of blood. Hemoglobin (Hb) was estimated by Sahli's hemoglobinometer (5). It was expressed as gram percent of blood. Packed cell volume was estimated by microhematocrit method (5). Total erythrocyte count and total leukocyte count were estimated following the method described by Jain (6) by using Gower's solution (SD fine-Chem. Limited, Mumbai) and WBC diluting fluid (Merck Limited, Mumbai) respectively. Differential leukocyte count was determined following the method described by Sastry (7). The data were subjected to analysis of variance for statistical interpretation (8).

Results and Discussion

The effect of sub-acute acetamiprid toxicity on mean values of TEC, Hb, PCV, MCV, MCH, MCHC and TLC along with relative counts of neutrophil, lymphocyte, eosinophil, monocyte and basophil for each experimental group is given in Table 1. The administration of acetamiprid orally for 28 days caused significant ($P \leq 0.05$) reduction in the levels of TEC of rats belonging to group III and IV compared to rats of control (group I). There were no significant ($P \leq 0.05$) differences in PCV, Hb and MCHC in rats of group III and group IV as compared to rats of control group. The mean values of MCV and MCH were found to be significantly ($P \leq 0.05$) higher in rats of group III and group IV as compared to control group. Though there were no significant differences in mean values of TEC, PCV, MCV and MCH in rats of group II, but a significant decrease in Hb and MCHC in rats of group II and TLC in rats of group IV was observed as compared to rats of control group. Significant ($P \leq 0.05$) increase in the mean values of percent neutrophil was found in the rats of group II, III and IV as compared to control group. However, there was significant decrease in percent lymphocyte count in the rats of group II, III and IV as compared to rats of control group. There were no significant differences on percent eosinophil, monocyte and basophil in acetamiprid administered rats as conpared to control group.

In the present study, acetamiprid, suspended in normal saline solution caused a statistically non-sig-

nificant increase ($P > 0.05$) in PCV level but significant decrease in RBC and hemoglobin, thus indicating that the insecticide could produce macrocytic hypochromic anaemia if administered orally for 28 days. This study also showed that acetamiprid caused a significant decrease in the levels of total white blood cells particularly in high dose group (group IV). The hematological examinations after 30 days exposure to monocrotophos in albino rats revealed decreases in Hb and RBC, indicating the pesticide induced anaemia (9). It has been suggested that compound having benzene ring or other ring structure acts as a hapten that combines with a protein constituent of leukocytes to form an antigen to which animal develops antibodies which is toxic to leukocytes causing either lysis or agglutination (10). Acetamiprid is also a ring structure compound and thus may have caused leukocytopenia. The acetamiprid might have also direct cytotoxic effect on leukocyte as also suggested in gentamicin toxicity in guinea pigs (11). It therefore showed that with continuous administration of this insecticide to animal, the principle function of phagocytes, which is to defend against invading microorganisms by ingesting and destroying them, thus contributing to cellular inflammatory processes, may be compromised (12). It should be noted that acetamiprid caused a significant decrease in the level of lymphocyte in all treated groups with significant increase in the level of neutrophils. Continuous exposure to this insecticide may then lead to lymphopaenia, which may have an immunosuppressive effect. The observed neutrophilia and lymphocytopenia might have occurred due to the adverse effect of insecticide on the normal functioning of bone marrow, stress (6) or due to varied factors responsible for the normal leukocyte balance (13).

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